Xenacoelomorpha's significance for understanding bilaterian evolution

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The Xenacoelomorpha, with its phylogenetic position as sister group of the Nephrozoa (Protostomia + Deuterostomia), plays a key-role in understanding the evolution of bilaterian cell types and organ systems. Current studies of the morphological and developmental diversity of this group allow us to trace the evolution of different organ systems within the group and to reconstruct characters of the most recent common ancestor of Xenacoelomorpha. The disparity of the clade shows that there cannot be a single xenacoelomorph 'model' species and strategic sampling is essential for understanding the evolution of major traits. With this strategy, fundamental insights into the evolution of molecular mechanisms and their role in shaping animal organ systems can be expected in the near future.

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Introduction: model systems and evolutionary biology

When writing about evolutionary biology and model systems, a contradiction becomes evident. Evolutionary research is a comparative science and has its foundation in examining the diversity of organisms that all carry information about their evolutionary past. In evolutionary biology, insights and understanding are gained by comparisons of as many species as possible. Contrary to this, model systems are chosen as exemplars that are ideal for investigations of a particular process and are thus easy to handle and accessible to a broad range of methods. Model systems are under detailed observation and in the spotlight of major efforts to find principles that possibly can lead to the formulation of general mechanisms. Well-known examples for animal developmental and cell biology models are the fruitfly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*, in which basic principles of developmental processes have been studied in great detail. It might be because the field of evolutionary developmental biology — EvoDevo — has its origin in developmental biology and not evolutionary biology that species under investigation are often called 'model species'. Criteria for selected representative species are primarily the ease of access to collected material and their ability to be cultivated in the lab [1]. In some cases, a supposedly larger number of ancestral characters or a dominant role in ecosystems have played an additional role in selecting model species. These arguments were used to attract sufficient funding for genome sequencing and developmental studies that are cost-intensive investigations. Several years ago, the focus on one species for each larger animal clade was reasonable, given the large effort necessary for collecting sufficient resources (e.g. genomic information) for developmental studies. Genome sequencing and the establishment of cDNA libraries were only affordable in collaborative efforts, and large amounts of embryonic material were needed to conduct molecular work. The evolutionary insights gained by studying only these model species are limited since morphological and developmental variation inside the groups is not considered. The recent inclusion of additional, even closely related, species into comparisons indicated in most cases that the developmental pathways are flexible and that findings in one species cannot be necessarily generalized for a clade over long evolutionary distances. The good news is that we can now go beyond the 'model system'-era of EvoDevo: Advances in sequencing technology and genome editing allow the implementation of advanced technology into new species much quicker and also much cheaper. The reconstruction of the animal phylogeny progresses in large steps and this allows a wiser choice of species to answer explicit questions about organ system and cell type evolution [2•]. Hypotheses based on only a handful of model systems can now be tested by the inclusion of more species, and this is finally strengthening the 'Evo' component of the field of 'EvoDevo'.

The Xenacoelomorpha (Figure 1) provides a case that illustrates well that the old approach of choosing a single 'model system' for gaining evolutionary insights will likely fail. In this review we outline the characteristics of the group and its importance for understanding animal evolution. We furthermore show that the diversity of the group reveals the independent evolution of characters

Figure 1

Current Opinion in Genetics & Development
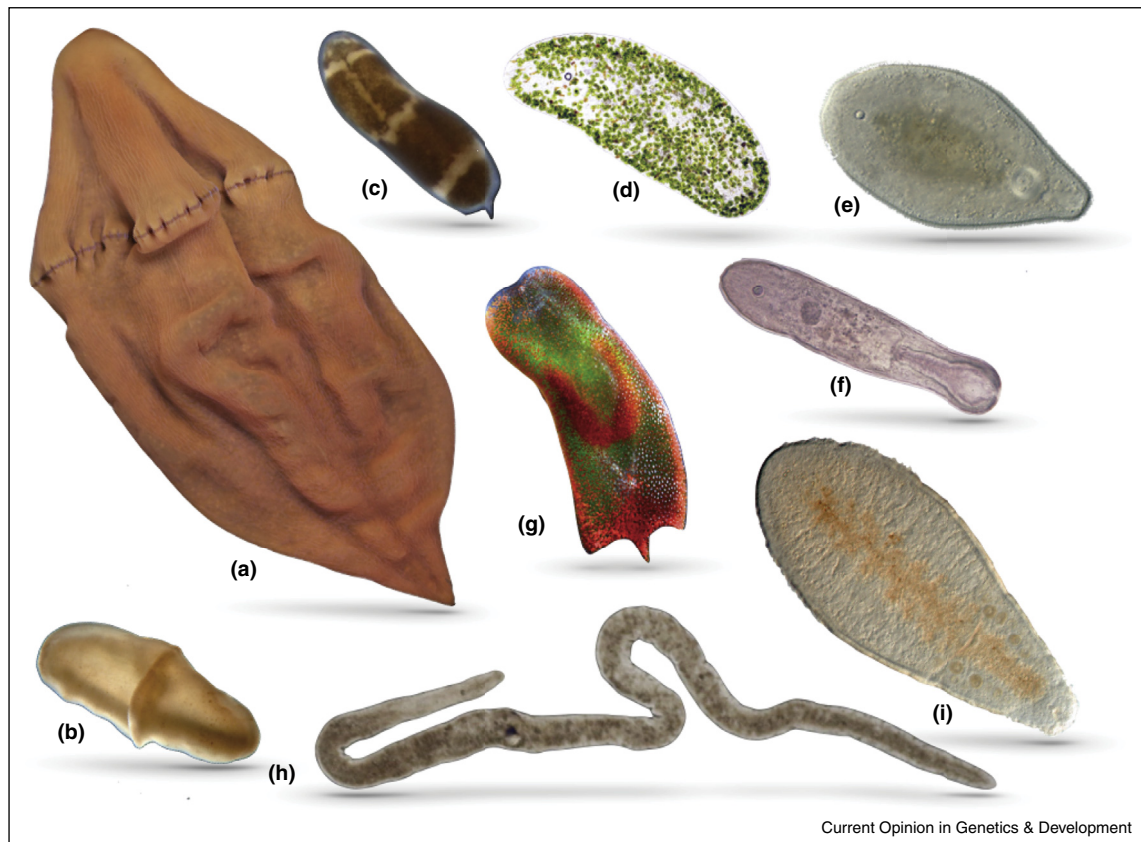
Disparity of Xenacoelomorpha. **(a)** *Xenoturbella profunda*, **(b)** *Xenoturbella bocki*, **(c)** *Hofstenia miamia* (Acoela), **(d)** *Symsagittifera roscoffensis* (Acoela), **(e)** *Isodiametra pulchra* (Acoela), **(f)** *Diopisthoporus psammophilus* (Acoela), **(g)** *Convolutriloba longifissura* (Acoela), **(h)** *Nemertinoides elongatus* (Nemertodermatida), **(i)** *Meara stichopi* (Nemertodermatida).
Source: Photos courtesy of Greg Rouse (xenoturbellas), Arthur Haug (*S. roscoffensis*), Erik Röttinger (*C. longifissura*), Ulf Jondelius (*D. psammophilus*, *H. miamia*, *N. elongatus*).

that have been seen as 'complex' when viewed from an anthropocentric or 'nephrozoan' perspective [3].

Xenacoelomorpha's significance

Their phylogenetic position and body composition makes the Xenacoelomorpha a key group to study when aiming to understand bilaterian evolution [4••]. The monophyly of the Xenacoelomorpha, comprised by *Xenoturbella*, Acoela, and Nemertodermatida, has been first supported by molecular evidence in 2009 [5]. One phylogenomic study suggested an affiliation of the Xenacoelomorpha with deuterostomes, albeit with only weak support [6]. The placement of Xenacoelomorpha as sister group to the Nephrozoa — Protostomia + Deuterostomia — has received solid support in recent molecular analyses [4••,5]. The morphology of the group is in line with this placement since they share some plesiomorphic characters with cnidarians (such as e.g. the single opening to the digestive tract [7]) but also possess characters that are seen as apomorphies of the Bilateria (e.g. bilateral symmetry, mesoderm, longitudinal and ring musculature)

[8••,9]. This composition of the xenacoelomorph body plan allows the determination of the sequence of evolution of bilaterian traits (Figure 2). However it becomes clear that only a detailed knowledge of the morphology and development of a large number of species from this taxon allows the discrimination between ancestral and derived characters and the reconstruction of the ground pattern. Recent progress has been made investigating a number of xenacoelomorph species regarding different aspects of their morphology and development, leading to a deeper understanding of this group and illustrating that it is unreasonable to focus on just one model species [10,11•,12–15,16•,17••,18•].

Significant characters xenacoelomorphs share with Cnidaria

The epidermis of xenacoelomorphs is completely ciliated, and they use these cilia to glide or swim, similar to the planula stage of Cnidaria. As in medusozoan cnidarians, the hatchlings of *Xenoturbella* and nemertodermatids do not possess a functional mouth opening and only later

Figure 2
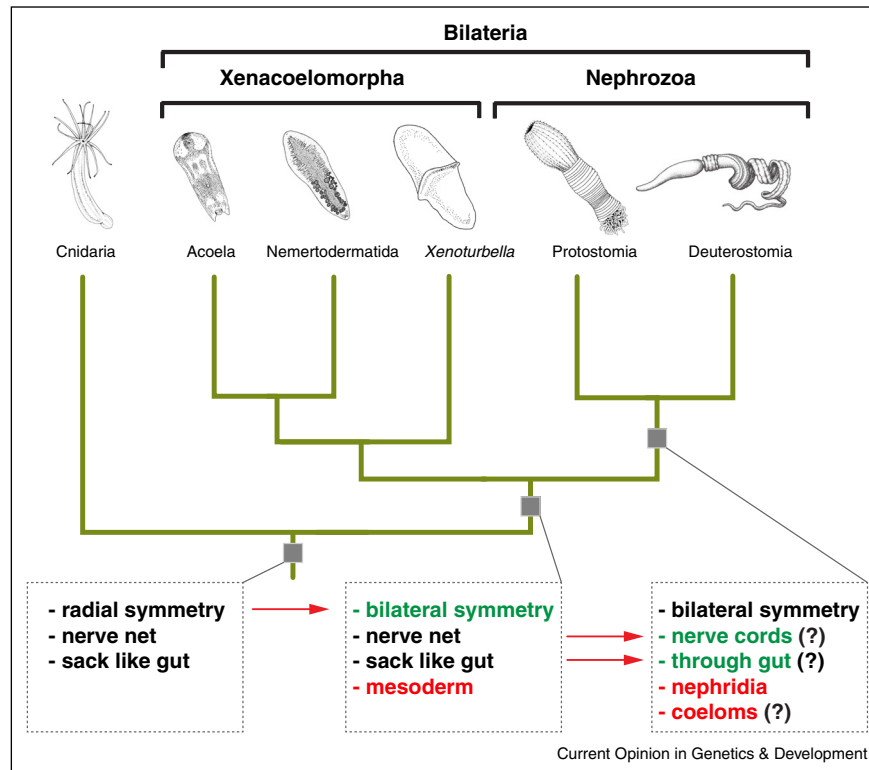
Phylogenetic relationships and the sequence of the evolution of bilaterian characters. Significant organ systems and their major transitions (red arrows) and novelties (red) mapped on the phylogeny.

develop the mouth to begin feeding [11[•],14,15]. Mature xenacoelomorphs have the mouth as the single opening [19] to a blind gut that is epithelial in *Xenoturbella* [20[•]] and Nemertodermatida, but has been modified as syncytial tissue in the Acoela [21]. This blind gut lacks gastric subdivisions (although the nemertodermatid *Meara stichopi* has branching gut tissue [11[•]]) and occupies large parts of the body. The digestive system is lined by non-epithelial gonads, and the oocytes are released either through the mouth opening or by body rupture [8[••]]. Some acoel groups of the Bursaria have evolved a new opening for the extrusion of fertilized oocytes and can possess specialized copulatory organs [22]. A basiepidermal nerve net can be reconstructed for the ground pattern of the Xenacoelomorpha since xenoturbellids lack any nervous system internalizations, as do most nemertodermatids [13,16[•]], but all xenacoelomorphs investigated so far possess a basiepidermal nerve net. Explicit excretory organs that conduct ultrafiltration are absent in cnidarians as well as in xenacoelomorphs, which renders nephridia — protonephridia and metanephridia — a novelty for the Nephrozoa. Recent comparative studies of the early development of nemertodermatids suggest that the duet-cleavage pattern, as it has been described for all acoel species so far, is a derived character and that likely a less stereotypic, regulative cleavage pattern similar to that of

Cnidaria is ancestral for the Xenacoelomorpha [11[•],23]. The shared characters of xenacoelomorphs and Cnidaria are the reason why this group has been described as 'simple' and similar to the early bilaterian stem species [8[••],24].

Significant characters xenacoelomorphs share with Nephrozoa

The characters that xenacoelomorphs share with protostomes and deuterostomes led to their designation as an 'intermediate' taxon, allowing the reconstruction of the sequence of the evolution of bilaterian traits [9]. The most evident similarity of the Xenacoelomorpha with the Nephrozoa is the bilateral symmetry, in which a clear left and right body side can be identified and in which the direction of movement is to the anterior [24]. Although anthozoan cnidarians are also bilaterally symmetric, here the identification of a left and right body is impossible and recent molecular studies could not determine a bona fide mechanism that could support the homology between any of the anthozoan and bilaterian body axes [25,26]. Here, investigations in xenacoelomorphs could provide deeper insights. A major innovation of the Bilateria was the mesodermal germ layer that likely evolved from the endoderm [27]. In acoelomorphs, the mesoderm seems to form the longitudinal and ring musculature of the body

and possibly the germ line and stem cell system [12,21]. The stem cell system of acoels is responsible for the growth and maintenance of the body [12,18•,28]. More detailed descriptions of acoel regeneration and the stem cell system can be found in a future edition of this journal (see Srivastava in Volume 40).

As in the remaining Bilateria, the acoelomorph mesoderm separates from the endomesoderm after gastrulation [23]. Furthermore, in both Xenaceolomorpha and Nephrozoa the endomesoderm gastrulates at the vegetal pole, which is the opposite side from that of a cnidarian embryo, which gastrulates at the animal pole [29]. The mechanism of this A-V inversion of the site of gastrualnion in the lineage to the Bilateria remains unclear.

## The sequence of the evolution of bilaterian characters

The chimeric nature of the Xenacoelomorpha in sharing important characters with cnidarians and nephrozoans has a tremendous impact on the understanding of the evolution of bilaterian characters on the morphological and molecular level. The phylogenetic position and the character distribution within the Xenacoelomorpha indicate that bilateral symmetry evolved before the evolution of the through gut [19,30] and that the first mesodermal derivative was musculature and not coeloms [12]. Furthermore, the bilaterian mouth that was likely not surrounded by an oral nerve ring, did not develop from the blastopore, and evolved before the anus [19]. The sequence of the evolution of these characters stand against recent narratives about the evolution of the Bilateria that are based on the enterocoely scenario of bilaterian evolution [31,32]. The sequence of the evolution of bilaterian characters will allow us to dissect the molecular mechanisms that triggered their evolution using explicit investigations of xenacoelomorph development. For example, the presence of only a nerve net in xenacoelomorphs will allow us to determine the ancient role of important nervous system patterning mechanisms that have been the subject of intense debates [33,34]. Because recent studies of xenacoelomorphs suggest that the first mesoderm was solely musculature, the question arises how new mesodermal cell types of the Bilateria evolved from a basic molecular developmental mechanism that originally triggered only muscle. Our understanding of the ancestral bilaterian body appearance is also affected: the xenacoelomorphs suggest it to be a rather small, benthic — maybe even interstitial — direct developing animal. Recent progress in resolving animal phylogenies support this view by rendering a small — possibly meiofaunal — species as most recent common ancestor for the large protostome clades Ecdysozoa and Spiralia [35–38]. This is of paleontological significance because small and soft-bodied species are not easily fossilized, which might have diminished the early bilaterian evolution from the paleontological tableau.

Evolution and variation within Xenacoelomorpha

Since all recent animal species diverged from their last common ancestor for the same time, it is not surprising to find many novelties and evolutionary modifications of major organ systems inside the xenacoelomorphs. The recent description of new *Xenoturbella* species that can be larger than 20 cm (Figure 1a) is broadening the biodiversity of the clade and extends their biogeographical distribution [17••]. The discovery also shows that *Xenoturbella* is cosmopolitan rather than a unique outlier found in Scandinavian fjords. There are interesting novelties and 'major' changes in the body plan that occurred within the Xenacoelomorpha (Figure 3), especially the Acoela showing a number of modifications of the ancestral state (Figure 3). Some acoels have gained an additional body opening — the female gonopore — to facilitate the release of gametes [22]. The digestive tract in Xenacoelomorphs has changed from an epithelial gut to a syncytial digestive system in the acoels, where the position of the mouth can vary from anterior (e.g. *Hofstenia*) to the far posterior (Diopisthoporidae), with most species having it positioned in the approximate middle of the body. The parenchymatic 'mesenchyme' seems to be a novel mesodermal tissue gained in the acoel lineage, which illustrates an interesting case of cell type evolution [12,21]. The nervous system in the Xenacoelomorpha underwent dramatic modifications that mirror the evolution of the nephrozoan nervous system: from an ancient nerve net at the base of the Xenacoelomorpha, several dorsal and ventral longitudinal basiepidermal condensations have been formed multiple times independently [10,11•,13,16•,39•,40,41]. In some lineages anterior condensations have been internalized to form a brain, and bundles of longitudinal nerves have been multiplied and internalized to form nerve cords [13,39•]. These modifications correlate with an elaboration of the behavioral complexity in the Acoela, such as swimming in three-dimensional interstitial environment, circadian rhythms, active predation, and swarm behavior [42,43].

Is a large step in nephrozoan evolution — the evolution of nerve cords and brains — only a small step for xenacoelomorphs? It seems that it is more the 'chordate'-view on evolution that makes the nerve cord and brain evolution in the Nephrozoa seemingly a big deal. Neglecting the modifications in different clades limits the perception of the evolution of animal body plans and makes small steps seem to be fundamental from the human perspective [3]. Initial genomic insights into the gene complement of *Xenoturbella bocki* (Figure 1b) and the acoel *Symsagittifera roscoffensis* (Figure 1d) show that *Xenoturbella* possesses a much larger gene complement of the Hox, Wnt, bHLH and GPCR families than the acoel species [39•,41]. In acoels, there is also a higher sequence divergence that correlates with the longer branches in phylogenetic analyses that are caused by faster evolutionary rates. In this

Figure 3
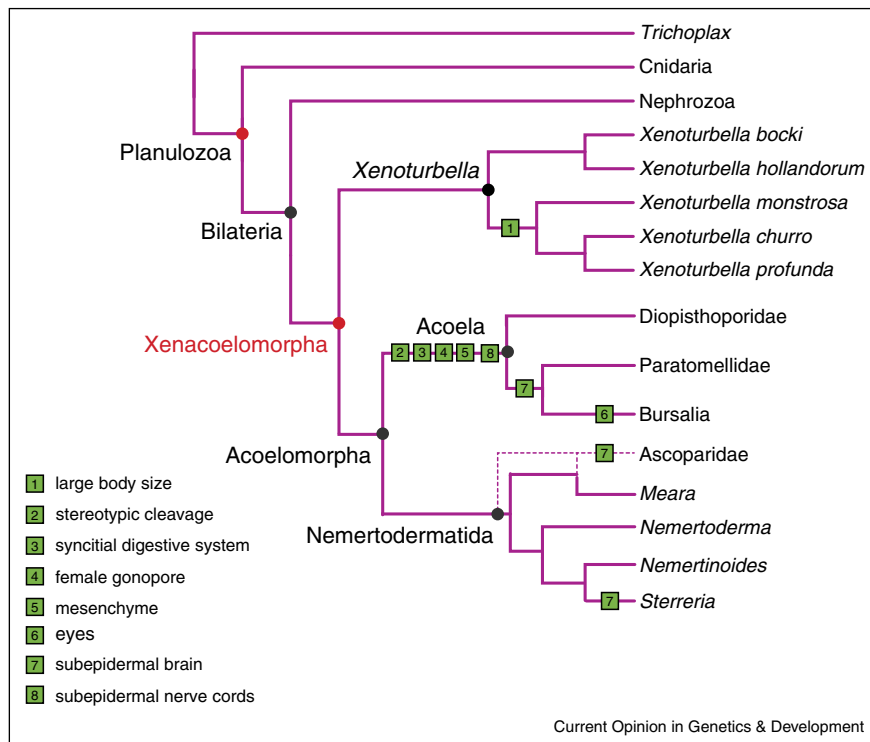
Character evolution within Xenacoelomorpha. Phylogenetic relationships based on recent molecular phylogenetic studies [4,17••,22,46]. Examples of character evolution inside the clade Xenacoelomorpha (outgroups not labeled).

Table 1

Advanced techniques and resources.

Xenoturbella	Illumina transcriptomes (Cannon *et al*., 2016 [4••]; Rouse *et al*., 2016 [17••])
Nemertodermatida	Illumina transcriptomes (Cannon *et al*., 2016 [4••])
Meara stichopi	Embryonic material (Børve and Hejnol, 2014 [11•])
Nemertoderma westbladi	Embryonic material (47)
Acoela	Illumina transcriptomes (Srivastava *et al*., 2014 [18•]; Cannon *et al*., 2016 [4••])
Isodiametra pulchra (breeding, Rieger *et al*., 1988)	Whole mount *in situ* hybridization (Hejnol and Martindale, 2008 [9])
Convolutriloba	Functional interference (RNAi) (48)
(breeding, Shannon and Achatz, 2007)	Microinjection (49)
Hofstenia miamia	Husbandry/breeding (50,51)
(breeding, Srivastava *et al*., 2014 [18•])	
Symsagittifera roscoffensis	

context it is interesting that — contrary to what has been found for parasitic bilaterians — the accelerated molecular evolution in the acoel lineage correlates with the gain of morphological specializations and not so much with their loss [4••,5,39•,41].

Future prospects in technique and resource development

The prospects for technique and resource development for xenacoelomorphs will not differ from most other animal species and is strongly correlated with the implementation of newest technologies (Table 1). The most limiting aspect is the access to embryonic material of different species. Although several acoel species can be cultured easily in the laboratory over generations, no *Xenoturbella* or nemertodermatid species has been cultured in the lab in a closed cycle.

Conclusions

Xenacoelomorphs provide an essential taxon for understanding bilaterian evolution, but their diversity makes it difficult to choose a single species as a 'model'. Previous xenacoelomorph species that have been declared as 'models', such as *Symsagittifera roscoffensis* [44] and *Isodiametra*

pulchra [10], show a very high number of derived characters. They are useful for studies of the internal evolution but an extrapolation to the whole group can lead to wrong conclusions about the homology of organ systems (i.e. conclusions of the brain homology between acoels and bilaterians [45]). Studies of the development and molecular architecture of organ systems need to consider the whole group to allow conclusions about evolution. Considering this, the selection of which exact species to investigate should be guided by the specific question.

Acknowledgements

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